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Ectodermal dysplasia–skin fragility syndrome resulting from a new atypical homozygous cryptic acceptor splice site mutation in *PKP1*

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Abbreviations: ectodermal dysplasia–skin fragility (EDSF), Plakophilin-1 (PKP1), polymerase chain reaction (PCR), reverse transcriptase-polymerase chain reaction (RT-PCR)

Keywords: *PKP1*, Plakophilin-1, ectodermal dysplasia–skin fragility syndrome, splice site mutation

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Ectodermal dysplasia–skin fragility (EDSF) syndrome (MIM604536) is an autosomal recessive disorder associated with loss-of-function mutations in *PKP1*, encoding the desmosomal protein plakophilin-1 [1]. Clinically, EDSF syndrome is characterized by skin fragility with generalized superficial erosions, patches of scale crust on the trunk and limbs, perioral cracking and inflammation, hypotrichosis, palmoplantar keratoderma with painful fissuring, and other more variable ectodermal anomalies [2, 3]. Recognized as the first genetic disorder of desmosome junctions, EDSF syndrome is now classified as a specific suprabasal form of autosomal recessive epidermolysis bullosa simplex [3]. Following the first report of EDSF syndrome in 1997 [1], only 13 cases have been reported so far. Here, we present a new case caused by a novel homozygous *PKP1* splice site mutation. The molecular pathology in this case is particularly noteworthy as the mutation occurs 9-base pairs upstream from an acceptor splice yet leads to a cryptic acceptor splice site with frameshift, loss of PKP1 expression, and the classic features of EDSF syndrome.

A 27 year-old Turkish female, born to consanguineous parents, had a history of lifelong trauma-induced skin fragility. On examination, she had cheilitis with perioral fissuring (Fig. 1a), hypotrichosis and scalp erosions (Fig. 1b), absence of axillary hair (Fig. 1c), and scattered blisters, erosions and crusts over the face and limbs (Fig. 1d). In addition, the patient had thickened finger-nails (Fig. 1e) and toe-nails (Fig. 1f) with subungual hyperkeratosis, and plantar keratoderma with fissuring (Fig. 1g). She had no problems sweating or with her teeth. No other family members were affected.

Histopathological examination from perilesional skin (arm) revealed hyperkeratosis, acanthosis, and dissociation between keratinocytes (See supplementary Fig. 1Sa). Immunofluorescence microscopy staining for plakophilin 1 showed a complete absence of labeling, and more diffuse intracytoplasmic staining for desmoplakin in contrast to the keratinocyte cell membrane fluorescence in control skin (See supplementary Fig. 1Sb). Transmission electron microscopy showed widening of intercellular spaces between keratinocytes, a reduced number of desmosomes, and markedly condensed tonofilaments with peri-nuclear retraction (See supplementary Fig. 1Sc).

Following informed consent, and in accordance with the Declaration of Helsinki principles, we performed Sanger sequencing on all coding exons and flanking intronic regions of *PKP1* (GenBank NM_001005337.2) using genomic DNA extracted from peripheral blood. A homozygous single nucleotide transition within intron 8, IVS8-9G>A, was identified. Since this location is outside most disease-associated splice site mutations, we initially assumed it was a non-functional polymorphism, although sequencing of *PKP1* failed to

identify any other clear loss-of-function mutations that could be compatible with the absence of PKP1 expression seen on skin microscopy. Moreover, we also found that both parents were heterozygous carriers for this intron 8 single nucleotide transition, and that two unaffected siblings had wild-type sequence (Fig. 2a).

With regard to IVS8-9G>A, this particular variant has not been described before in the Human Gene Mutation Database, Single Nucleotide Database (http://www.ncbi.nlm.nih.gov/projects/SNP/), nor in the 1000 Genomes database (http://www.1000genomes.org/), nor in the Exome Aggregation Consortium (http://exac.broadinstitute.org/) files. Surprisingly, analysis using the *in silico* splicing prediction programs Human Splicing Finder and Fruitfly predicted a new cryptic acceptor splice site for IVS8-9G>A in *PKP1* [4, 5].

The consequences of this splice site mutation on *PKP1* expression were investigated by reverse transcriptase (RT)-PCR analysis using RNA extracted from unwounded patient skin. RNA extraction was performed using the RNAeasy Plus Universal Mini kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. The Super-Script™ III First-Strand Synthesis System for RT-PCR (Invitrogen, Carsbad, CA, USA) was used to reverse-transcribe RNA to cDNA. The cDNA was amplified using primers (5'-ggtggaccctgaggtcttct-3' and 5'-caggggcagtcatagttgt-3') to obtain a product covering exon 8-9 of *PKP1* cDNA. Sequencing of the RT–PCR fragment (using these primers) showed an insertion of 7 nucleotides before exon 9 (Fig. 2b), that leads to a frameshift and a downstream premature termination codon (p.Ser471Serfs*32; Fig. 2c). Thus the molecular basis of EDSF syndrome in this individual appears to be homozygosity for IVS8-9G>A in *PKP1*.

This aberrant splicing mutation in *PKP1* is unusual. Approximately 10% of disease-causing mutations are located within splice site sequences at exon-intron junctions, with most splice-site mutations occurring at the +1 or +2 positions for donor sites or the -1 or -2 positions for acceptor sites [6]. In EDSF syndrome, 14 different pathogenic mutations in *PKP1* have been reported, which comprise 3 nonsense, 3 frameshift, and 8 splice site mutations (Fig. 2c). The 8 splicing mutations are all intronic, including 3 located at the +1 donor, 2 at the -1 acceptor, and 3 in the -2 acceptor positions. A pathogenic mutation at the -9 position is therefore noteworthy, not only for *PKP1*. Intronic mutations that lead to cryptic splicing are not ultra rare in genetic diseases, although it is easy to initially dismiss a nucleotide change at the -9 position as being non-pathogenic. Thus, our case serves as an important reminder of the spectrum of the less obvious splice site mutations that can form the molecular basis of some diseases. For our *PKP1* mutation, cDNA sequencing indicates

that the mutation induces a cryptic acceptor splice site, leading to an addition of 7 base pairs to the coding sequence which causes a premature termination codon 32 amino acids downstream.

Our patient with EDSF syndrome has similar clinical manifestations and skin pathology to most of the other cases of this desmosomal genodermatosis. PKP1 has an important role in providing stable cohesion to keratinocytes throughout the spinous layer to resist mechanical stress [7], as well as contributing to tight junction biology with implications for the control of cell and tissue growth [8]. Moreover, PKP1 has dynamic roles in cell signaling, for example through interactions with proteins such as focal adhesion kinase [9]. Collectively, these recent observations – and other cell biology data – provide further insight into understanding the complex pathobiology of EDSF syndrome. From a molecular genetics perspective, however, it is important to appreciate that seemingly innocuous mutations outside consensus splice sites can have markedly deleterious effects on gene function that may only be fully appreciated by *in silico* analyses and assessment of gene expression.

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Figure Legends

Fig 1. Clinical features of EDSF syndrome.

The patient displays cheilitis with perioral fissuring (a), erosions and sparse scalp hair (b), absence of axillary hair (c), erosions and crusts over the elbow (d), thickened finger-nails (e) and toe-nails (f) with subungual hyperkeratosis, and plantar keratoderma with fissuring (g).

Fig 2. Molecular studies of the *PKP1* splicing mutation

(a) Sanger sequencing of *PKP1* reveals a homozygous mutation (IVS8-9G>A) in the proband's genomic DNA, while both parents are heterozygous carriers and two siblings are wild-type. (b) Sequencing of the cDNA across the exon8/9 border shows the insertion of 7 nucleotides before exon 9. (c) Schematic diagram of the splicing mutation (d) Database of mutations in *PKP1* in EDSF syndrome. Previously described gene mutations are indicated in black and the novel mutation in red. Double arrows indicate homozygous mutations; Joined arrows depict compound heterozygous mutations. Colored circled numbers 1 through 9 refer to the armadillo-domains of the PKP1 protein.